Soil Biology and Biochemistry 135 (2019) 13–19

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Warming increases microbial residue contribution to soil organic carbon in an alpine meadow

Xueli Ding[a], Shengyun Chen[b], Bin Zhang[a], Chao Liang[c], Hongbo He[c], William R. Horwath[d,*]

[a] Jiangsu Key Laboratory of Agricultural Meteorology, School of Applied Meteorology, Nanjing University of Information Science & Technology, Nanjing, 210044, China
[b] Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou, 730000, China
[c] Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, 110016, China
[d] Department of Land, Air and Water Resources, University of California, Davis, CA, 95616, USA

ARTICLE INFO

Keywords:
Microbial necromass
Amino sugar
Warming
C sequestration
Tibet plateau

ABSTRACT

The contribution of microbial residues to soil organic carbon (SOC) is a process highly influenced by soil properties. We evaluated the presence of microbial amino sugar residues in soil (0–50 cm) of control and warmed plots in an alpine meadow on the Qinghai-Tibet Plateau. Alpine grasslands in the Qinghai-Tibet Plateau store large amounts of soil C and are highly vulnerable to climate change. Results showed that warming significantly increased total microbial residues across the 0–50 cm soil depth. The proportion of microbial-derived C to SOC significantly increased in warmed plots (52% on average) by soil depth compared to the control (38%). Higher microbial turnover and selective preservation into organo-mineral complexes likely explains the observed result. Given insignificant change in total SOC, our results infer an alteration of the SOC source configuration (microbial-derived vs. plant-derived). The observed greater magnitude of warming effects on fungal residues compared to bacterial illustrate a distinct community response to warming. We conclude that warming has the potential to influence soil C sequestration through increased microbial residue inputs, consequently altering its composition and source configuration. Our work provides valuable insights at the molecular level to identify mechanisms of microbial-mediated C processes that are influenced by climate change in high elevation ecosystems.

1. Introduction

Climate change is expected to result in a global increase of 1.8–4.0 °C by the end of this century, with a greater warming occurring in higher latitudinal and altitudinal ecosystems (IPCC, 2014). The Qinghai-Tibet plateau, highest and largest plateau in the world, has experienced rapid climate warming with an average temperature increase of 0.2 °C per decade in the past 50 years (Chen et al., 2013). The area stores large amounts of soil organic carbon (SOC, ~33.5 Pg in the top 75 cm profile within an area of 1,627,000 km^2) (Wang et al., 2002). The loss of SOC in these sensitive ecosystems may lead to the loss of significant CO_2 and emission of greenhouse gases (Schuur et al., 2015). The impact of warming on microbial processes will likely affect the ability and capacity to store soil C (Bardgett et al., 2008; Schimel and Schaeffer, 2012).

Microorganisms are depicted as gatekeepers for terrestrial C fluxes through catabolism and anabolism (Liang et al., 2015). In particular, the importance of microbial residues as precursors to SOC and their ability to influence its stability has been increasingly recognized (Simpson et al., 2007; Miltner et al., 2012; Cotrufo et al., 2013). Amino sugars are examples of microbial residues representing cell wall components (Amelung, 2001). Amino sugars are stable against fluctuations in microbial biomass size and primarily occur as microbial necromass (Guggenberger et al., 1999; Glaser et al., 2004). They can provide a time-integrated indicator of microbial contributions to soil C storage (Ding et al., 2013; Struecker and Joergensen, 2015). The study of climate warming effects on soil microorganisms has focused primarily on microbial biomass size and turnover, community structure and enzyme activities (Allison and Treseder, 2008; Zhang et al., 2015; Xiong et al., 2016). Microbial residues are accepted as a major source of SOC, but uncertainties remain on the effects of soil warming on changing input amounts and quality (Liang and Balser, 2012; Throckmorton et al., 2015).

Climate warming will affect soils initially at the surface, and then gradually to subsurface soil by heat flux downward and changes in plant and microbial residue inputs (Fierer et al., 2003). Subsoil contains

* Corresponding author.
E-mail address: wrhorwath@ucdavis.edu (W.R. Horwath).

https://doi.org/10.1016/j.soilbio.2019.04.004
Received 22 October 2018; Received in revised form 29 March 2019; Accepted 5 April 2019
Available online 10 April 2019
0038-0717/ © 2019 Published by Elsevier Ltd.

substantial quantities of C with long turnover times (Rumpel and Kögel-Knabner, 2011). In the Qinghai-Tibetan Plateau, greater than 47% of SOC is stored in subsoils (> 30 cm) (Yang et al., 2008). These subsoils have distinct resource availability gradients and microbial communities compared to topsoil (Eilers et al., 2012). It is unclear how the distribution of microbial residues and their contributions to soil C pool will change with depth under warming.

Our study addresses the uncertainty in the source of microbial residues (fungal vs bacterial) in response to soil warming and how this affects total soil C in a climate sensitive high elevation meadow ecosystem. We tested the following predictions: (1) Given that plant inputs into soil in the form of litter and root exudates generally increase with warming, it could increase catabolic and anabolic activities of soil microorganisms (Wild et al., 2014; Gunina and Kuzyakov, 2015), together with our previous work that shows an increase in both total microbial biomass and abundance in microbial communities with increasing temperature (Zhang et al., 2015), we therefore expect that warming could stimulate microbial residue accumulation and increase their potential contribution to the SOC pool. This effect may be particularly pronounced in subsoil where occurrence of labile C is scarce and dominated by stabilized organic matter compounds (Rumpel and Kögel-Knabner, 2011). (2) Generally, bacterial density and metabolic rates respond rapidly to warming (Ylla et al., 2014; Freixa et al., 2017; Bernabé et al., 2018), with bacteria often prevailing over fungi in subsoil due to oxygen limitations and restricted availability of fresh plant inputs (Struecker and Joergensen, 2015). Hence, we predict that the net increase of bacterial residues and their contribution to SOC would exceed those of fungal counterparts under warming conditions.

2. Materials and methods

2.1. Site description

The study site is located at the Beiluhe Observation and Research Station of Chinese Academy of Sciences (34°51′ N, 92°56′ E) on the Qinghai-Tibet Plateau (Fig. 1). The regional climate is continental with mean annual air temperature of −3.8 °C and mean annual precipitation of 383 mm. Precipitation falls mainly during the summer monsoon season. Frozen soil occurs from September to April. Soils are classified as Gelic Arenosols in the WRB soil classification system (IUSS Working Group WRB, 2006). The biome of this site is an alpine meadow dominated by *Kobresia pygmaea* C.B.Clarke (Cyperaceae), *K. robusta* Maxim. (Cyperaceae) and *Androsace tapete* Maxim. (Primulaceae).

2.2. Experimental design

To increase the soil temperature, open-top chambers (OTCs) were set up on the alpine meadow within a fenced area of 30 m × 30 m in late September 2008. The OTCs serve as passive warming devices according to the 'International Tundra Experiment' (ITEX) (Marion et al., 1997). Three warmed plots were covered throughout the year by OTCs which were truncated cones with a height of 0.4 m and bottom and top diameters of 1.48 and 1.08 m, respectively (Fig. 1). The OTCs were made of 1-mm thick translucent fiberglass (Sun-Lite® HP, Solar Components Corporation, Manchester, NH, USA). Parallel control plots were established at a distance of 2 m apart from the warmed plots. Dataloggers with automatic temperature sensors were used to record soil temperatures at depths of 10, 20, 30, 40 and 50 cm of both warmed and control plots on a half-hourly basis throughout the experiment. Soil temperature was increased by 1.4, 1.4, 1.1, 0.3 and 0.1 °C at depths of 10, 20, 30, 40 and 50 cm (Zhang et al., 2015).

2.3. Soil sampling and analysis

Soils were collected from the control and warmed plots after three years of the experiment. Seven randomized soil cores (3.14 cm in diameter) per plot were taken from the 0–50 cm depth with a hand auger and cut into five segments of 0–10, 10–20, 20–30, 30–40 and 40–50 cm. The depth segments within the same plot were combined, passed through a 2-mm sieve and then air-dried. Soil organic C was determined by the Walkley-Black procedure (Nelson and Sommers, 1996). Soil samples (between 0.5 and 1 g for surface and 2–3 g for subsurface) were oxidized by heating with potassium dichromate and concentrated H_2SO_4 at 135° C for 30 min. Excess dichromate was titrated with ferrous sulfate to determine organic C content. Basic soil properties at different depths of the warmed and control plots were adopted from Zhang et al. (2015) and presented in Table 1.

2.4. Amino sugar analysis

Soil amino sugars were determined according to the procedure of Zhang and Amelung (1996). Briefly, amino sugars were extracted, purified and converted to aldononitrile acetates, and the derivatives were separated on an Agilent 6890 A gas chromatograph equipped with a HP-5 fused silica column and flame ionization detector (Agilent Technologies, Santa Clara, CA, USA). Amino sugars were identified by comparison with standard peaks and quantified based on the internal standard (myo-inositol). Fungal C and bacterial C as an index for fungal residues and bacterial residues was calculated according to the method as described in Sradnick et al. (2014). Fungal C was calculated by subtracting bacterial glucosamine from total glucosamine, assuming that muramic acid and glucosamine occur at a 1–2 M ratio in bacterial cells: mmol fungal C g^{-1} dry weight = (mmol GlcN − 2 × mmol MurN) × 9 (Engelking et al., 2007). Bacterial C was calculated by multiplying the concentration of muramic acid by 45 (Appuhn and Joergensen, 2006). Microbial residue C was estimated as the sum of fungal and bacterial C.

2.5. Statistical analysis

A general linear model with Tukey's HSD as post hoc was used to test the effect of warming and depth on fungal and bacterial C as well as their ratios and proportions to soil organic C in the R program (version 3.2.4).

3. Results

3.1. Accumulation patterns of fungal and bacterial residues in soil

Warming significantly increased total microbial residues by 17.7% across soil depths (Table 2). Total microbial residues were significantly higher in surface soils (0–10 and 10–20 cm) than subsurface soils (20–30, 30–40 and 40–50 cm) (Table 2). Bacterial residues were significantly influenced by soil depth, but not by warming or their interaction (Table 2). Fungal residues were significantly influenced by warming and soil depth, but not by their interaction (Table 2). On average, the warmed soils contained 32.2% higher fungal residues than the control soils across all depths (Table 2). The depth trend of both bacterial and fungal residues mirrored that of total microbial residues (Table 2). The ratio of fungal to bacterial residues was significantly higher in the warmed than control plots of the alpine meadow, but generally similar across soil depths with minor exception (Table 2).

3.2. Microbial residue-derived C normalized to SOC

Experimental warming had no apparent effect on total SOC content (10.5 and 9.33 mg g^{-1} as averaged across the 0–50 cm soil layers under control and warmed plots, respectively) (Table 1). However, warming significantly increased the proportions of fungal residues by 53.4% across the 0–50 cm soil depth (Table 3) but had little effect on the proportions of bacterial residues (Table 3). The proportion of total microbial residues to SOC was significantly influenced by warming, but

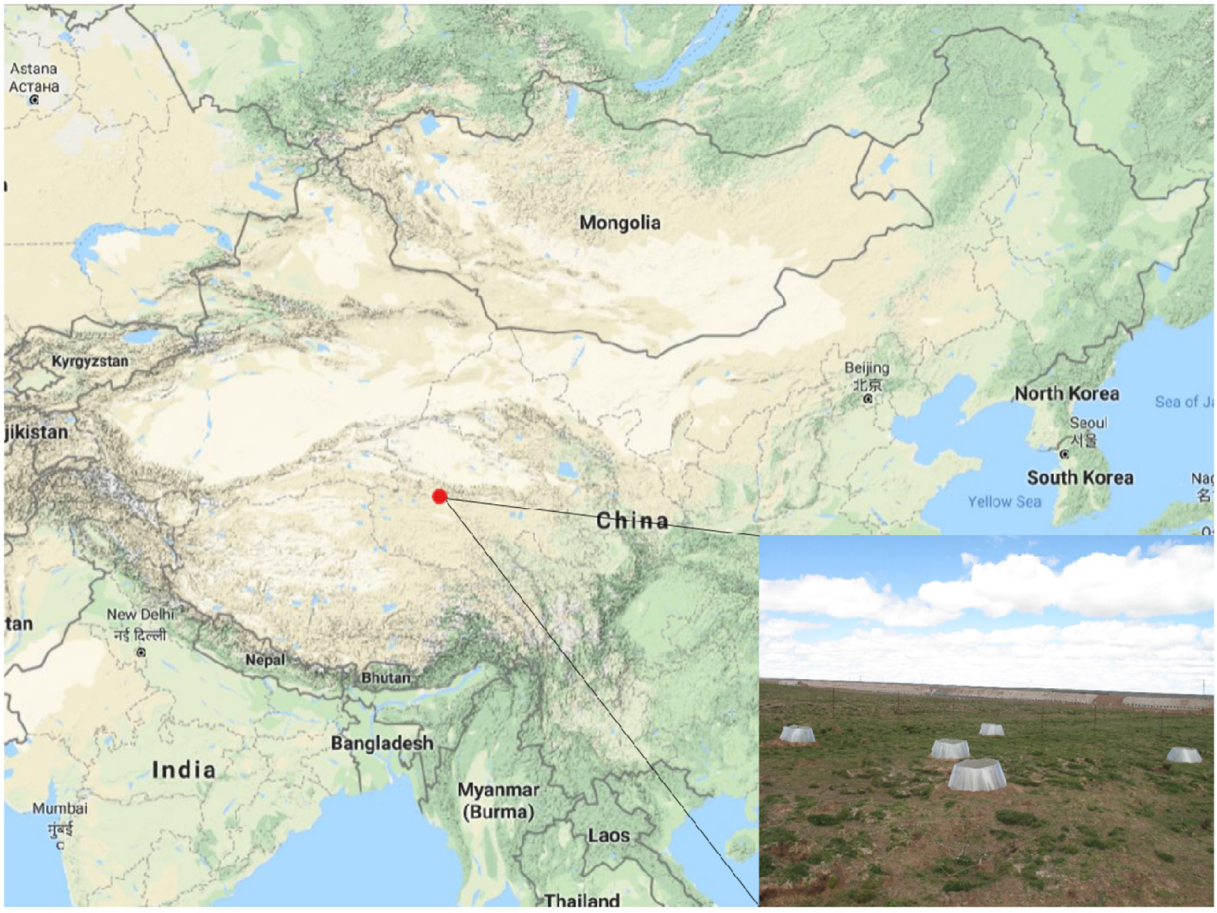

Fig. 1. Site location and field plots.

Table 1
Basic soil properties in control and warmed plots of an alpine meadow at five depth intervals.

Depth (cm)	Treatment	Soil organic C (mg g^{-1})	Total N (mg g^{-1})	C/N	pH	Soil moisture (%)
0–10	Control	16.5 (1.32)	1.25 (0.11)	13.2 (2.17)	8.69 (0.13)	37.8 (0.92)
	Warming	17.1 (3.26)	1.23 (0.26)	14.0 (5.61)	8.73 (0.28)	35.8 (1.26)
10–20	Control	13.5 (1.83)	1.16 (0.08)	11.6 (2.35)	8.80 (0.10)	43.7 (1.21)
	Warming	11.1 (1.71)	0.98 (0.07)	11.3 (2.61)	8.80 (0.14)	41.9 (0.65)
20–30	Control	9.93 (1.17)	0.94 (0.14)	10.6 (2.88)	8.80 (0.11)	43.5 (0.68)
	Warming	7.28 (0.73)	0.70 (0.03)	10.5 (1.53)	8.81 (0.17)	42.1 (0.33)
30–40	Control	7.43 (1.21)	0.72 (0.04)	10.3 (2.33)	8.97 (0.04)	46.8 (0.84)
	Warming	6.67 (1.30)	0.66 (0.02)	10.1 (2.32)	8.87 (0.21)	44.9 (0.86)
40–50	Control	5.97 (0.95)	0.66 (0.10)	9.02 (2.81)	9.08 (0.16)	47.8 (0.41)
	Warming	5.13 (1.70)	0.55 (0.13)	9.13 (5.15)	8.89 (0.22)	46.1 (0.69)
Source of variance						
Treatment	F	4.059	8.413	0.017	0.596	5.132
	P	0.058	**0.009**	0.896	0.449	**0.031**
Depth	F	44.682	29.544	14.066	2.521	18.249
	P	< **0.001**	< **0.001**	< **0.001**	0.073	< **0.001**
Treatment × depth	F	0.929	0.837	0.214	0.495	0.825
	P	0.467	0.518	0.928	0.74	0.583

Note: numbers in the parentheses are standard errors; data was adopted from Zhang et al. (2015).

not by soil depth or their interaction (Table 3). The proportion of total microbial residues to SOC was 37.9% higher in the warmed than the control plots (Table 3).

4. Discussion

4.1. Warming altered microbial residue accumulation in the alpine meadow soils

As climate change progresses, the sequestration and maintenance of SOC is of great importance in climate sensitive ecosystems like the Qinghai-Tibet Plateau. In our study, warming significantly stimulated the accumulation of microbial residues within 50-cm soil layer, which is

Table 2
Concentration and ratio of microbial residues at profiles of soils under control and warming in an alpine meadow on the Qinghai-Tibet Plateau.

Soil depth (cm)	Total microbial residues (mg C g^{-1})			Bacterial residues (mg C g^{-1})			Fungal residues (mg C g^{-1})			Fungal/bacterial residues		
	Control	Warming	Mean	Control	Warming	Mean	Control	Warming	Mean	Control	Warming	Mean
0–10	6.22 (0.53)	7.71 (0.25)	6.97a	2.53 (0.09)	2.91 (0.31)	2.72a	3.70 (0.56)	4.81 (0.21)	4.25a	1.47 (0.24)	1.70 (0.22)	1.58a
10–20	6.06 (0.74)	6.94 (0.60)	6.50a	2.95 (0.36)	2.82 (0.49)	2.82a	3.11 (0.41)	4.12 (0.17)	3.61a	1.06 (0.07)	1.53 (0.20)	1.29 ab
20–30	3.53 (0.11)	3.53 (0.48)	3.53b	1.95 (0.32)	1.46 (0.06)	1.46b	1.58 (0.22)	2.07 (0.42)	1.82b	0.88 (0.23)	1.39 (0.23)	1.14 ab
30–40	2.59 (0.16)	3.37 (0.06)	2.98b	1.47 (0.18)	1.85 (0.30)	1.85b	1.12 (0.03)	1.52 (0.24)	1.32b	0.79 (0.11)	0.90 (0.24)	0.84b
40–50	1.86 (0.18)	2.29 (0.27)	2.08b	0.95 (0.18)	1.08 (0.24)	1.08b	0.91 (0.03)	1.21 (0.05)	1.06b	1.05 (0.24)	1.22 (0.24)	1.14 ab
Mean	4.05 A	4.77 B		1.97 A	2.02 A		2.08 A	2.75 B		1.05 A	1.35 B	
Source of variance	F	P		F	P		F	P		F	P	
Warming	7.912	**0.011**		0.093	0.763		13.122	**0.002**		5.025	**0.037**	
Depth	58.872	< **0.001**		15.701	< **0.001**		48.754	< **0.001**		3.263	**0.033**	
Warming × depth	0.934	0.464		0.868	0.5		0.822	0.526		0.377	0.822	

Numbers in the parentheses are standard errors. Different lower-case letters indicate significant differences among soil depths. Different upper-case letters indicate significant differences between control and warmed plots.

consistent with our first predication. This result suggests that warming might facilitate microbial growth and proliferation under climate warming and lead to the increased input of microbial-derived C in soils overtime. Soil organic C is allocated for respiratory energy production (catabolism) or towards biosynthetic stabilization (anabolism) such as cell maintenance and growth during microbial decomposition of organic matter (Bradford, 2013; Hagerty et al., 2014). Given that warming had minimal impact on total SOC content in this study, the higher microbial residues therefore imply that microorganisms tend to invest more C in anabolism than catabolism in the warmer soil and/or that microbial residues are better preserved compared to other inputs.

In contrast to our study, Liang and Balser (2012) reported that microbial residues (indicated by amino sugar biomarkers) significantly declined after a 9-year warming treatment in a California annual grassland ecosystem in a Mediterranean climate. Soil moisture is believed to be a key factor driving the differential responses of microbial residue accumulation to warming between different studies (Sardans et al., 2008; Brockett et al., 2012). Generally, water content within warmed plots is lower in comparison with unwarmed plots, which possibly led to a decrease in microbial residue production through lower microbial growth in the dry California grassland (Liang and Balser, 2012). Warming-induced declines in water content may be not the limiting factor influencing microbial residue accumulation in our study, as moisture content in the warmed plot remained relatively high ranging from 35.8% to 46.1% (Zhang et al., 2015). In addition, the method of warming is different for these two studies: while a passive warming device was used to increase temperature in our study, a constant heat treatment (+1 °C) was applied at the soil surface in the study of Liang and Balser (2012). Further, the duration of warming is also different (3 and 9 years, respectively). All these can contribute to differences between these two studies. Given the role of microbial residues in SOC formation and stabilization, the microbial-derived C inputs will likely become significant in terms of C storage in soils of the Tibetan alpine grasslands in the context of climate warming.

Previous studies showed that warming alters soil properties and/or the quality of organic C sources, which would affect nutrient cycling (Hoover et al., 2012; Zhou et al., 2012). These changes often affect the size and structure of soil microbial communities (Zhang et al., 2015; Xiong et al., 2016). It can be expected that warming would influence the types of microbial residue inputs. Here, we found that warming significantly increased fungal residues at all soil depths to 50 cm, indicating greater sensitivity of fungal rather than bacterial groups to the warming, in contrast to our predication. Nevertheless, our result was consistent with the previous observation of higher phospholipid fatty acid estimated fungal biomass in the warmed than control plots in the same site (Zhang et al., 2015). This phenomenon is most likely related to the increased plant material input triggered by warming given that fungal contribution to microbial communities could be stimulated by litter input fresh as well as more root exudates, in particular in subsoil (Sanaullah et al., 2016).

Fungal residue enrichment with elevated temperature may affect future C balances as fungal residues are thought to be more persistent in soils than bacterial counterparts (Six et al., 2006; Amelung et al., 2008). Noteworthy, bacterial residues were less influenced by warming, leading to higher ratios of fungal to bacterial residues. We explained this observation as a relative preferential degradation of bacterial

Table 3
Proportion of total microbial residues, fungal and bacterial residues in soil organic C (SOC) at different soil depths under control and warming in an alpine meadow on the Qinghai-Tibet Plateau.

Soil depth (cm)	Total microbial residues (%SOC)			Fungal residues (%SOC)			Bacterial residues (%SOC)		
	Control	Warming	Mean	Control	Warming	Mean	Control	Warming	Mean
0–10	37.6 (1.76)	46.1 (4.26)	41.8a	22.1 (2.32)	28.7 (2.71)	25.4 ab	15.4 (1.15)	17.4 (2.31)	16.4a
10–20	46.2 (8.80)	66.6 (2.64)	56.4a	23.8 (5.05)	40.1 (3.77)	31.9a	22.4 (3.87)	26.5 (1.20)	24.4a
20–30	35.8 (2.19)	48.4 (6.27)	42.1a	16.3 (3.18)	28.3 (5.58)	22.3b	19.5 (2.41)	20.2 (1.10)	19.9a
30–40	35.3 (3.43)	52.2 (7.27)	43.8a	15.3 (1.69)	22.6 (1.64)	19.0b	20.0 (2.67)	29.6 (8.53)	24.8a
40–50	35.0 (2.53)	48.4 (10.1)	41.7a	17.2 (0.49)	25.4 (4.73)	21.3b	17.7 (3.01)	23.0 (5.92)	20.3a
Mean	38.0 A	52.4 B		18.9 A	29.0 B		19 A	23.3 A	
	F	P		F	P		F	P	
Warming	16.07	< **0.001**		20.826	< **0.001**		3.031	0.097	
Depth	2.488	0.076		4.123	**0.013**		1.59	0.216	
Warming × depth	0.314	0.865		0.678	0.614		0.39	0.814	

Numbers in the parentheses are standard errors. Different lower-case letters indicate significant differences among soil depths. Different upper-case letters indicate significant differences between control and warmed plots.

residues than fungal ones in the context of nutrient limitation (Ding et al., 2011; He et al., 2011). In the alpine grassland of Qinghai-Tibet Plateau, nitrogen is a limiting factor due to temperature limited nitrogen mineralization and plant competition (Zhou et al., 2014). Jia et al. (2017) reported that in the warmed plots more degradable SOC potentially intensified nitrogen limitation. He et al. (2011) stated that the preferential decomposition of bacterial residues served as the "capacitor" to compensate for N limitation. The preferential degradation of bacterial residues to meet N nutrient demand for plant and/or microbial growth could counteract the accumulation of bacterial residues. These results may also reflect differences in growth and turnover rate of microbes where fungi tend to be more dynamic given their niche and mode of growth while a significant proportion of bacterial cells may reside in protected or isolated areas. For example, bacteria often exist in protected niches such as soil aggregates and experience less external environmental fluctuations in micro-climate than fungi (Denef et al., 2001; Xiong et al., 2016).

4.2. Depth-related responses of microbial residue C to warming

Fungal and bacterial residues were much higher at 20 cm and above depths than at lower depths in both control and warmed plots, indicating quantitative differences in top- and subsoil. The differences should be attributed to changes in the availability of fresh plant material (Müller et al., 2016) and contrasting physicochemical conditions with depth (Struecker and Joergensen, 2015). The importance of vertical spatial heterogeneity in shaping the distribution of microbial residues has been reported in several studies (Fierer et al., 2003; Sradnick et al., 2014). Warming stimulated microbial residue accumulation at both top- and subsoil depths, inducing greater differences of total microbial residue changes along soil profile compared to the control. For example, a net increase in total microbial residues up to 23.9% for surface layers (0–10 cm) in warmed soils compared to the control treatment was observed. It is easy to understand this observation since increased new C sources through litter or root turnover and rhizodeposition with warming will increase allocation of more resources to belowground microbial growth and may accommodate a larger population of microorganisms (Högberg and Read, 2006; Ding et al., 2013).

Interestingly, we found that microbial residues within the 20–30 cm layer were not significantly affected by warming, while an increased abundance of microbial residues under warming was observed in deeper soil layers (30–40 cm and 40–50 cm). A possible explanation for this increase could be vertical transport of microbial cell materials from upper layers via precipitation (Liang and Balser, 2008). This could be facilitated by bonding of amino and amide-N group to clay particles in deeper soil (Lowe, 1983). Water table fluctuation are probably responsible for depth distribution of microbial residue C because a significant correlation was found between total microbial residues and soil moisture ($r = 0.85$, $p = 0.002$). The water table fluctuations affect the distribution of aerobic and anaerobic microbes (Morris et al., 2004) and consequently the accumulation of microbial residues. Our findings also illustrate that the presence of microbial-derived C in subsoils is not homogeneously distributed but spatially distinct. This heterogeneous distribution of microbial residues should be taken into account, when investigating microbial carbon sequestration in deep soil horizons.

4.3. Significance of microbial residue contribution to SOC under warming

The percentage of microbial-derived residue input to SOC maintenance and accumulation is fundamental to understand soil C biogeochemical process and for broad-scale estimates to validate global C modelling parameters (Liang and Balser, 2012). We calculated the proportion of microbial residues contributing to SOC and found that total microbial residues contributed approximately to 38% and 52% to SOC in control and warmed soils, respectively. Comparable proportions were reported in a tropical forest ecosystem (> 40%, Zhang et al., 2016), in an arable system (50% in the topsoil, Struecker and Joergensen, 2015) and in arable and grassland soils (50%, Khan et al., 2016). This percentage was also verified by studies using different methodologies, such as NMR spectroscopy (Simpson et al., 2007), application of ^{13}C labeled bacteria to soil (Miltner et al., 2012), and analysis of density fractions of SOM by in-source pyrolysis-field ionization mass spectrometry (Ludwig et al., 2015). This indicates that warming preferentially promoted the accumulation of microbial-derived C fractions in SOC pools relative to other C-inputs. This supports the role of microorganisms in continuously and gradually 'transferring' new microbial-derived compounds that play a significant role in a soil's capacity to sequester and stabilize SOC (Liang et al., 2017). In light of non-significant variations of total SOC stocks between warmed and control plots, our results also inferred that the relative proportion of microbial-versus plant-derived C have been altered by climate warming, resulting in a potential change in SOC composition, which may affect its quality. Microbial residues have been suggested as important components of the stable C pool in soils (Guggenberger et al., 1999; Glaser et al., 2004) via physical protection or a lack of activation energy due to chemical composition (Schweigert et al., 2015). The significant enrichment of microbial residues in SOC therefore have ecologically important implications in terms of long-term C storage in soil of the Qinghai-Tibetan Plateau.

It was noteworthy to observe the increase in the percentage of microbial residues under increased temperature below 30 cm (16.9% for 30–40 cm depth and 13.4% for 40–50 cm depth), in agreement with our predication that the warming effect on microbial residues would be more pronounced in subsoil. Given that soil depths below 10 cm had a lower SOC content regardless of treatment, we suggest warming triggers a greater priming effect in the deeper soil likely due to the lower available C (Rumpel and Kögel-Knabner, 2011). In this case, the net increase in microbial residues reveals that warming can intensify anabolic metabolism, resulting in more microbial biomass (Zhang et al., 2015) and promoting higher turnover and necromass accumulation as observed in our study. Importantly, given the higher clay content in subsoil, microbial residues may be better preserved by their interaction with mineral surfaces which can provide physical protection (Lehmann et al., 2007). Similarly, Rumpel and Kögel-Knabner et al. (2011) reported that products of microbial synthesis are a principle C source in mineral-organic associations in subsoils.

Given that subsoils (> 30 cm) contain almost half of SOC on the Qinghai-Tibetan Plateau (Yang et al., 2008), our results infer that the response of subsoil microbial C to warming will be pivotal in understanding the feedback of microbial mediation of terrestrial C cycling. Therefore, soil depth-related microbial C should be considered in predicting changes in C cycling or model development in the context of global change in climate sensitive ecosystems such as the Qinghai-Tibet meadow ecosystem. We propose that climate warming could significantly impact SOC quality and its stability but is depth dependent, likely due to substantial changes in the proportions of microbial-versus plant-derived C input to SOC stocks as a result of warming. As reported, microbial residues, representing a part of the stable soil C pool has a critical role as a C sink within the global C cycle, and thus even a small change in this pool has a large consequence for altering atmospheric composition (Liang et al., 2015).

In this study, we calculated microbial residue C from soil amino sugar data to estimate the contribution of microbial residues to soil organic matter by using conversion factors from Appuhn and Joergensen (2006) and Engelking et al. (2007). Other microbial products such as extracellular polymeric substances, enzymes and possibly secondary compounds would also be a significant source of microbial inputs (Joergensen and Wichern, 2018). The amino sugar conversion is widely used in studies used to support our results, for example Amelung et al., (2008); He et al., (2011); Liang et al., (2015). It assumes that other cellular components persist along with the amino sugars to contribute equally to SOM maintenance and soil C sequestration. This

could lead to an under- or overestimation of the contribution of the various components of microbial biomass to soil C. Therefore, care must be exercised when utilizing these conversion values, especially in modelling studies. Nevertheless, the converted values can serve as an independent check to estimate the relationships between microbial residues and SOC (Joergensen, 2018), and thus to compare relative changes in microbial residue contributions among different treatments.

In summary, our work revealed that warming significantly influenced microbial residue accumulation in the 50 cm profile of an alpine meadow ecosystem on the Qinghai-Tibet Plateau. This result illustrates global climate warming may induce positive feedback of microbial anabolic capacity and/or activity, which may directly impact microbial contributions to SOC via accelerating the transformation of both microbial-derived and soil C. The significant increase in fungal/bacterial residues and fungal residue/SOC ratios under the warmed treatment indicates that warming can increase the contribution of fungi to SOC pools. The change in microbial residue contribution to SOC infers a potential change in SOM composition and likely quality since microbial residues represent a significant source of stable C. These findings can provide valuable insights to capture the molecular composition and dynamics of SOC that is driven by microbial activity and turnover, which will deepen our recognition of the nature and mechanisms of microbial-mediated C process in response to global climate change. We also emphasize the need to take the soil depth-related microbial residue C into account in developing C cycling models and predicting C feedbacks in future global change scenarios.

Acknowledgement

The authors would like to thank the Beiluhe Observation and Research Station staff on Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences. This work was financially supported by the Natural Science Foundation of China (41690142, 41571237) and the National Key Research and Development Program (2017YFD0200100).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2019.04.004.

References

Allison, S.D., Treseder, K.K., 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. Global Change Biology 14, 2898–2909.
Amelung, W., 2001. Methods using amino sugars as markers for microbial residues in soil. In: Lal, R., Kimble, J.M., Follett, R.F., Stewart, B.A. (Eds.), Assessment Methods for Soil Carbon. CRC/Lewis Publishers, Boca Raton, FL, pp. 233–270.
Amelung, W., Brodowski, S., Sandhage-Hofmann, A., Bol, R., 2008. Combining biomarker with stable isotope analyses for assessing the transformation and turnover of soil organic matter. Advances in Agronomy 100, 155–250.
Appuhn, A., Joergensen, R.G., 2006. Microbial colonisation of roots as a function of plant species. Soil Biology and Biochemistry 38, 1040–1051.
Bardgett, R.D., Freeman, C., Ostle, N.J., 2008. Microbial contributions to climate change through carbon cycle feedbacks. The ISME Journal 2, 805–814.
Bernabé, T.N., de Omena, P.M., dos Santos, P.V., de Siqueira, V.M., de Oliveira, V.M., Romero, G.Q., 2018. Warming weakens facilitative interactions between decomposers and detritivores, and modifies freshwater ecosystem functioning. Global Change Biology 24, 3170–3186.
Bradford, M.A., 2013. Thermal adaptation of decomposer communities in warming soils. Frontiers in Microbiology 4, 333.
Brockett, B.F.T., Prescott, C.E., Grayston, S.J., 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. Soil Biology and Biochemistry 44, 9–20.
Chen, H., Zhu, Q.A., Peng, C.H., Wu, N., Wang, Y.F., Fang, X.Q., Gao, Y.H., Zhu, D., Yang, G., Tian, J.Q., Kang, X.M., Piao, S.L., Ouyang, H., Xiang, W.H., Luo, Z.B., Jiang, H., Song, X.Z., Zhang, Y., Yu, G.R., Zhao, X.Q., Gong, P., Yao, T.D., Wu, J.H., 2013. The impacts of climate change and human activities on biogeochemical cycles on the Qinghai-Tibetan Plateau. Global Change Biology 19, 2940–2955.
Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The microbial efficiency-matrix stabilization MEMS framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable organic matter? Global Change Biology 19, 988–995.
Denef, K., Six, J., Bossuyt, H., Frey, S.D., Elliott, E.T., Merckx, R., Paustian, K., 2001. Influence of dry–wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biology and Biochemistry 33, 1599–1611.
Ding, X.L., Han, X.Z., Zhang, X.D., Qiao, Y.F., 2013. Effects of contrasting agricultural management on microbial residues in a Mollisol in China. Soil and Tillage Research 130, 13–17.
Ding, X.L., He, H.B., Zhang, B., Zhang, X.D., 2011. Plant-N incorporation into microbial amino sugars as affected by inorganic N addition: a microcosm study of ^{15}N-labeled maize residue decomposition. Soil Biology and Biochemistry 43, 1968–1974.
Eilers, K.G., Debenport, S., Anderson, S., Fierer, N., 2012. Digging deeper to find unique microbial communities: the strong effect of depth on the structure of bacterial and archaeal communities in soil. Soil Biology and Biochemistry 50, 58–65.
Engelking, B., Flessa, H., Joergensen, R.G., 2007. Shifts in amino sugar and ergosterol contents after addition of sucrose and cellulose to soil. Soil Biology and Biochemistry 39, 2111–2118.
Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. Soil Biology and Biochemistry 35, 167–176.
Freixa, A., Acuna, V., Casellas, M., Pecheva, S., Romaní, A.M., 2017. Warmer night-time temperature promotes microbial heterotrophic activity and modifies stream sediment community. Global Change Biology 23, 3825–3837.
Glaser, B., Turrion, M.B., Alef, K., 2004. Amino sugars and muramic acid– biomarkers for soil microbial community structure analysis. Soil Biology and Biochemistry 6, 399–407.
Guggenberger, G., Frey, S.D., Six, J., Paustian, K., Elliott, E.T., 1999. Bacterial and fungal cell-wall residues in conventional and no–tillage agroecosystems. Soil Science Society of America Journal 63, 1188–1198.
Gunina, A., Kuzyakov, Y., 2015. Sugars in soil and sweets for microorganisms: review of origin, content, composition and fate. Soil Biology and Biochemistry 90, 87–100.
Hagerty, S.B., van Groenigen, K.J., Allison, S.D., Hungate, B.A., Schwartz, E., Koch, G.W., Kolka, R.K., Dijkstra, P., 2014. Accelerated microbial turnover but constant growth efficiency with warming in soil. Nature Climate Change 4, 903–906.
He, H., Zhang, W., Zhang, X., Xie, H., Zhuang, J., 2011. Temporal responses of soil microorganisms to substrate addition as indicated by amino sugar differentiation. Soil Biology and Biochemistry 43, 1155–1161.
Högberg, P., Read, D.J., 2006. Towards a more plant physiological perspective on soil ecology. Trends in Ecology & Evolution 21, 548–554.
Hoover, S.E., Ladley, J.J., Shchepetkina, A.A., Tisch, M., Gieseg, S.P., Tylianakis, J.M., 2012. Warming, CO_2, and nitrogen deposition interactively affect a plant pollinator mutualism. Ecology Letters 15, 227–234.
IPCC, 2014. Fifth Assessment Report, Climate Change 2014: Impacts, Adaptation, and Vulnerability. . http://www.ipcc.ch/report/ar5/wg2/.
IUSS Working Group WRB, 2006. World Reference Base for Soil Resources-A Framework for International Classification, Correlation and Communication. World Soil Resources Report No 103. FAO, Rome.
Jia, J., Feng, X., He, J., He, H., Lin, L., Liu, Z., 2017. Comparing microbial carbon sequestration and priming in the subsoil versus topsoil of a Qinghai-Tibetan alpine grassland. Soil Biology and Biochemistry 104, 141–151.
Joergensen, R.G., 2018. Amino sugars as specific indices for fungal and bacterial residues in soil. Biology and Fertility of Soils 54, 559–568.
Joergensen, R.G., Wichern, F., 2018. Alive and kicking: why dormant soil microorganisms matter. Soil Biology and Biochemistry 116, 419–430.
Khan, K.S., Mack, R., Castillo, X., Kaiser, M., Joergensen, R.G., 2016. Microbial biomass, fungal and bacterial residues, and their relationships to the soil organic matter C/N/P/S ratios. Geoderma 271, 115–123.
Lehmann, J., Kinyangi, J., Solomon, D., 2007. Organic matter stabilization in soil microaggregates: implications from spatial heterogeneity of organic carbon contents and carbon forms. Biogeochemistry 85, 45–57.
Liang, C., Balser, T.C., 2008. Preferential sequestration of microbial carbon in subsoils of a glacial-landscape toposequence, Dane County, WI, USA. Geoderma 148, 113–119.
Liang, C., Balser, T.C., 2012. Warming and nitrogen deposition lessen microbial residue contribution to soil carbon pool. Nature Communications 3, 1222.
Liang, C., Gutknecht, J., Balser, T.C., 2015. Microbial lipid and amino sugar responses to long-term simulated global environmental changes in a California annual grassland. Frontiers in Microbiology 6, 385.
Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology 2, 17105.
Ludwig, M., Achtenhagen, J., Miltner, A., Eckhardt, K.U., Leinweber, P., Emmerling, C., Thiele-Bruhn, S., 2015. Microbial contribution to SOM quantity and quality in density fractions of temperate arable soils. Soil Biology and Biochemistry 81, 311–322.
Lowe, L.E., 1983. Carbohydrates in soil. In: Schnitzer, M., Khan, S.U. (Eds.), Soil Organic Matter. Elsevier, New York, pp. 65–93.
Marion, G.M., Henry, G.H.R., Freckman, D.W., Johnstone, J., Jones, G., Jones, M.H., Lévesque, E., Molau, U., Mølgaard, P., Parsons, A.N., Svoboda, J., Virginia, R.A., 1997. Open-top designs for manipulating field temperature in high-latitude ecosystems. Global Change Biology 3, 20–32.
Morris, D.R., Glaz, B., Daroub, S.H., 2004. Organic matter oxidation potential determination in a periodically flooded histosol under sugarcane. Soil Science Society of America Journal 68 (3), 994–1001.
Miltner, A., Bombach, P., Schmidt-Brücken, B., Kästner, M., 2012. SOM genesis: microbial biomass as a significant source. Biogeochemistry 111, 41–55.
Müller, K., Kramer, S., Haslwimmer, H., Marhan, S., Scheunemann, N., Butenschon, O., Scheu, S., Kandeler, E., 2016. Carbon transfer from maize roots and litter into bacteria and fungi depends on soil depth and time. Soil Biology and Biochemistry 93, 79–89.

Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter. In: Methods of Soil Analysis, Part 3, Chemical Methods, Book Series No. 5. Soil Science Society of America and American Society of Agronomy, Madison, pp. 961–1010.
Rumpel, C., Kögel-Knabner, I., 2011. Deep soil organic matter-a key but poorly understood component of terrestrial C cycle. Plant and Soil 338, 143–158.
Sanaullah, M., Chabbi, A., Maron, P.A., Baumann, K., Tardy, V., Blagodatskaya, E., Kuzyakov, Y., Rumpel, C., 2016. How do microbial communities in top-and subsoil respond to root litter addition under field conditions? Soil Biology and Biochemistry 103, 28–38.
Sardans, J., Penuelas, J., Estiarte, M., 2008. Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. Applied Soil Ecology 39, 223–235.
Schimel, J.P., Schaeffer, S.M., 2012. Microbial control over carbon cycling in soil. Frontiers in Microbiology 3, 1–11.
Schuur, E.A.G., McGuire, A.D., Schädel, C., Grosse, G., Harden, J.W., Hayes, D.J., Hugelius, G., Koven, C.D., Kuhry, P., Lawrence, D.M., Natali, S.M., Olefeldt, D., Romanovsky, V.E., Schaefer, K., Turestsky, M.R., Treat, C.C., Vonk, J.E., 2015. Climate change and the permafrost carbon feedback. Nature 520, 171–179.
Schweigert, M., Herrmann, S., Miltner, A., Fester, T., Kästner, M., 2015. Fate of ectomycorrhizal fungal biomass in a soil bioreactor system and its contribution to soil organic matter formation. Soil Biology and Biochemistry 88, 120–127.
Simpson, A.J., Simpson, M.J., Smith, E., Kelleher, B.P., 2007. Microbially derived inputs to soil organic matter: are current estimates too low? Environmental Science and Technology 41, 8070–8076.
Six, J., Frey, S.D., Tiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70, 555–569.
Sradnick, A., Oltmanns, M., Raupp, J., Joergensen, R.G., 2014. Microbial residue indices down the soil profile after long-term addition of farmyard manure and mineral fertilizer to a sandy soil. Geoderma 226–227, 79–84.
Struecker, J., Joergensen, R.G., 2015. Microorganisms and their substrate utilization patterns in topsoil and subsoil layers of two silt loams, differing in soil organic C accumulation due to colluvial processes. Soil Biology and Biochemistry 91, 310–317.
Throckmorton, H.M., Bird, J.A., Monte, N., Doane, T., Firestone, M.K., Horwath, W.R., 2015. The soil matrix increases microbial C stabilization in temperate and tropical forest soils. Biogeochemistry 122, 35–45.
Wang, G., Qian, J., Cheng, G., Lai, Y., 2002. Soil organic carbon pool of grassland soils on the Qinghai-Tibetan Plateau and its global implication. The Science of the Total Environment 291, 207–217.
Wild, B., Schnecker, J., Alves, R.J.E., Barsukov, P., Barta, J., Capek, P., Gentsch, N., Gittel, A., Guggenberger, G., Lashchinskiy, N., Mikutta, R., Rusalimova, O., Santruckova, H., Shibistova, O., Urich, T., Watzka, M., Zrazhevskaya, G., Richter, A., 2014. Input of easily available organic C and N stimulates microbial decomposition of soil organic matter in arctic permafrost soil. Soil Biology and Biochemistry 75, 143–151.
Xiong, Q.L., Pan, K.W., Zhang, L., Wang, Y.J., Li, W., He, X.J., Luo, H.Y., 2016. Warming and nitrogen deposition are interactive in shaping surface soil microbial communities near the alpine timberline zone on the eastern Qinghai-Tibet Plateau, southwestern China. Applied Soil Ecology 101, 72–83.
Yang, Y., Fang, J.Y., Tang, Y.H., JI, C.J., Zheng, C.G., He, J.S., Zhu, B., 2008. Storage, patterns and controls of soil organic carbon in the Tibetan grasslands. Global Change Biology 14, 1592–1599.
Ylla, I., Canhoto, C., Romaní, A.M., 2014. Effects of warming on stream biofilm organic matter use capabilities. Microbial Ecology 68, 132–145.
Zhang, B., Chen, S.Y., Zhang, J.F., He, X.Y., Liu, W.J., Zhao, Q., Zhao, L., Tian, C., 2015. Depth-related responses of soil microbial communities to experimental warming in an alpine meadow on the Qinghai-Tibet Plateau. European Journal of Soil Science 66, 496–504.
Zhang, W., Cui, Y., Lu, X., Bai, E., He, H., Xie, H., Liang, C., Zhang, X., 2016. High nitrogen deposition decreases the contribution of fungal residues to soil carbon pools in a tropical forest ecosystem. Soil Biology and Biochemistry 97, 211–214.
Zhang, X., Amelung, W., 1996. Gas chromatographic determination of muramic acid, glucosamine, mannosamine, and galactosamine in soils. Soil Biology and Biochemistry 28, 1201–1206.
Zhou, J.Z., Xue, K., Xie, J.P., Deng, Y., Wu, L.Y., Cheng, X.H., Fei, S.F., Deng, S.P., He, Z.L., Van Nostrand, J.D., Luo, Y.Q., 2012. Microbial mediation of carbon-cycling feedbacks to climate warming. Nature Climate Change 2, 106–110.
Zhou, L., Song, M.H., Wang, S.Q., Fan, J.W., Liu, J.Y., Zhong, H.P., Yu, G.R., Gao, L.P., Hu, Z.M., Chen, B., Wu, W.X., Song, T., 2014. Patterns of soil ^{15}N and total N and their relationships with environmental factors on the Qinghai-Tibetan Plateau. Pedosphere 24, 232–242.